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EARLY T LYMPHOCYTE PROGENITORS

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ABSTRACT

The earliest steps along the pathway leading to T cells in mice and humans are reviewed. These are the steps between the multipotent hemopoietic stem cell (HSC) and the fully committed precursors undergoing T cell receptor (TCR) gene rearrangement. At this level significant differences between adult and fetal lymphopoiesis have been demonstrated. The extent of lymphoid commitment of precursors within bone marrow is still unresolved, although HSCs clearly undergo developmental changes before migration to the thymus. Both multipotent and T-restricted precursors have now been isolated from fetal blood, suggesting both may seed the thymus. Within the thymus, several minute but discrete populations of T precursors precede the stage of TCR gene rearrangement. They include precursors that are not exclusively T-lineage committed, although they are distinct from HSCs. These precursors have a potential to form NK cells, B cells, dendritic cells, and sometimes other myeloid cells. Some factors that control early lymphoid development are discussed, including IL-7 and the Ikaros transcription factors. These will eventually help to clarify the process of T-lineage commitment.

INTRODUCTION

All lymphoid cells derive, ultimately, from multipotent hemopoietic stem cells (HSCs) found in the liver during fetal life and then in the bone marrow of

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the adult. Because such stem cells can be considered as "early T lymphocyte progenitors," we must define the territory of this review. We begin calling stem cells "early lymphoid progenitors" when they have lost at least some of their capacity to form blood cells other than lymphocytes. We cease calling T lymphocyte progenitors "early" once they have completed rearrangement of the genes specifying either the β or the γ chain of a T cell antigen receptor. We therefore review data on a range of progenitor cells from those that have been called lymphoid stem cells to those termed pro-T cells. Our interest is in the process of T lymphocyte lineage commitment.

DIFFERENCES BETWEEN FETAL AND ADULT T LYMPHOPOIESIS

The lymphocytes formed in adult life are derived from different sets of HSCs than those formed in the embryo. This is most evident for T cell development in the thymus where the site of lymphoid development is well separated from the source of stem cells. The classic studies of Le Douarin and colleagues (1, 2) demonstrated that the avian thymus is colonized in succession by discrete waves of stem cells. A similar situation occurs in the fetal mouse thymus, where Jotereau et al (3) have shown that the stem cells or lymphoid precursors seeding the fetal thymus between days 10 and 13 are the source of the T cells produced by the thymus up to the first week after birth, while the stem cells seeding the thymus after day 13 of fetal life produce a second generation of T cells that begins to displace or dilute the first generation from day 7 after birth. Further seeding of the thymus occurs in adult life (4, 5).

The implications of these differences in stem cell origin for subsequent lymphopoiesis would be minimal unless the fetal and adult lymphoid developmental programs differ. Such a possibility was happily ignored by many workers who used the analysis of thymuses harvested on successive days of embryonic life to deduce the successive steps of both embryonic and adult T cell development. In the main this optimistic approach was effective; most of the key events are common to the embryo and the adult. However, recent work has highlighted some important differences. The striking finding is that some of these reflect precommitment or restrictions on developmental options at the level of an otherwise multipotent HSCs.

The clearest example is $\gamma\delta$ T cell generation in which during ontogeny successive waves of $\gamma\delta$ T cells exit the thymus, destined to seed different peripheral tissues. The successive waves are characterized by differing T cell antigen receptor (TCR) gene utilization (6). In embryonic mice the first wave of TCR-gene rearrangement in the thymus leads to Vγ3-bearing cells, which

become the Thy-1^+ dendritic epidermal $\gamma\delta$ cells found in the skin of the adult (7). Ikuta et al (8) have shown, by purifying HSCs from fetal liver or adult bone marrow and using these to repopulate fetal or adult thymuses, that $V\gamma3^+$ T cell development requires not only a fetal thymus environment, but also fetal HSCs. Adult stem cells have apparently lost the potential to form $V\gamma3^+$ T cells. The basis of this altered developmental potential has not yet been elucidated.

Another difference between embryonic and adult lymphoid development is the presence of the enzyme terminal deoxynucleotide transferase (TdT) in early T and B lineage cells of the adult but not of the embryo. This enzyme is responsible for template-independent (N region) nucleotide addition during antigen-receptor gene rearrangement; this introduces an additional level of diversity into both the TCR and immunoglobulin (Ig) repertoire (9–11). Lymphoid cells produced during adult life have this additional N region diversity, while lymphoid cells produced during embryonic life do not; however, adult mice lacking TdT because of a mutated gene have an "immature lymphocyte repertoire" with few N nucleotides (10, 11). When fetal thymus cultures were reconstituted with adult or with embryonic stem cell sources, both allowed $V\gamma4^+$ T cell development, but the $V\gamma4$-$J\gamma1$ transcripts from the adult precursors had modified junctions with N nucleotide insertion (12). The generality of this result still needs formal testing for other TCR genes. However, it is now reasonable to propose (13) that the ability to express TdT and perform N nucleotide insertions represents another difference in developmental potential already determined at the multipotent HSC level. Many other differences between adult and embryonic lymphopoiesis may be secondary consequences of this difference in TdT expression.

One possible candidate is the propensity of adult thymic $CD4^-8^-$ precursor cells to generate many more $CD4^+$ than $CD8^+$ T cells, in contrast to early embryonic $CD4^-8^-$ precursors where the T cell progeny are produced in nearly equal levels. Adkins (14) has demonstrated, by seeding embryonic precursors into adult thymuses and vice versa, that the ratio of $CD4^+$ to $CD8^+$ progeny is determined by the precursor cells themselves rather than by the thymic environment. She has suggested these precursor cell differences reflect, in turn, differences in the successive waves of stem cells populating the thymus (14). Since both CD4 and CD8 T cells are produced only after a very stringent selection for immunological specificity, it is possible that the difference in relative production reflects the presence or absence of TdT in the precursor cells. By this argument the higher N region TCR diversity associated with the adult TdT^+ precursors in some way leads to more class-II major histocompatibility complex–restricted receptors appropriate for $CD4^+$ T cells.

EARLY T PROGENITORS AND STEM CELLS IN BONE MARROW

The HSCs of adult mouse and human bone marrow are heterogeneous in capacity to produce long-term reconstitution of the hematopoietic system as well as in cell cycle status, physical properties, and surface antigen expression (13, 15–18). The stem cell population appears to be separable into subpopulations representing stages of differentiation between the most primitive long-term reconstituting cells and the lineage-committed progenitors (18). A major issue is whether commitment to a given lymphoid lineage is an abrupt process or whether there exist intermediate self-renewing stem cells that are common to all lymphoid lineages, but no longer able to form myeloid or erythroid cells. Such a "common lymphoid-restricted" stem cell has been an almost mythical entity, prominent in wall-charts of hemopoiesis but elusive when its isolation from bone marrow is attempted.

The surface markers that have proven useful for the positive characterization of multipotent HSCs (Sca-1^+ Thy 1.1^{lo} c-kit^+ in the mouse; $CD34^+$ in the human) are also expressed by immature cells of various lineages. To obtain pure stem cells it is necessary to combine positive sorting for these markers with a procedure to exclude cells bearing lineage-restricted surface markers (Lin^+ cells). However, one might hope to isolate a lineage-restricted stem cell on the basis of early surface expression of one of these lineage markers. Although stem cell activity had been reported in the "Lin^+" fraction of mouse bone marrow, a careful reexamination of the finding has indicated that almost all long-term reconstituting activity is within the Sca-1^+ Thy 1.1^{lo} and Lin^- population (17–19). Perhaps this is not an absolute phenotype, since some stem cell activity is detected in Mac-1^{lo} and $CD4^{lo}$ fractions of normal mouse marrow (17, 19), and $CD4^{lo}$ Gr-1^{lo} progenitors have been detected in 5-fluorouracil–treated marrow (20). However, these stem cells bearing low levels of the lineage markers all appear to be multipotent.

It is possible that some markers other than those recognized by the usual "anti-Lin" cocktail of antibodies could serve to delineate a lymphoid-restricted stem cell. For example, the ER-MP12 antigen is on murine bone marrow cells with thymus repopulating activity (21), but it is not yet clear whether multipotent HSCs also bear this antigen. The Joro 37-5 and Joro 75 antibodies recognize Thy 1^+ Lin^- T-progenitor cells in murine bone marrow, but these appeared to be T-lineage restricted and unable to produce B cells (22). The antigen Sca-2, which is present on T precursors within the thymus (see later), but absent from long-term repopulating bone marrow HSCs, has been used by Antica et al (23) to search for thymus-colonizing cells in bone marrow. Although a new Sca-2^+ T-precursor population distinct from most HSCs was isolated, it was not

lymphoid restricted in its reconstitution potential. This result fits with evidence that the multipotent stem cells with rapid (3 wk) thymus colonizing activity after intravenous transfer are those more active cells with a high capacity to take up rhodamine 123 ($Rh123^{hi}$), and not the more quiescent long-term repopulating $Rh123^{lo}$ bone marrow HSCs (24, 25). Overall, these studies suggest that murine HSCs do undergo distinct phenotypic changes before leaving the bone marrow to seed the thymus. The extent of T commitment in bone marrow is not yet clear, but to date no population has been isolated with a capacity to make T and B lymphocytes but not myeloid or erythroid cells. Although the search for a lymphoid-committed stem cell in murine bone marrow has been unrewarding, it should be noted that even traces of primitive, multipotent HSCs in a lymphoid-committed precursor fraction would be enough to obscure evidence for lymphoid restriction.

Studies on adult human bone marrow have shown more promise in delineating a population of lymphoid-restricted precursors. Extending earlier evidence (26) that the $CD34^+$ fraction includes lymphoid progenitors, Galy et al (27) have subdivided the $CD34^+$ Lin^- population. Additional markers were employed, including Thy 1 and CD45RA, the latter chosen because early thymocytes are $CD45RA^+$. The developmental potential of the subpopulations of $CD34^+$ Lin^- cells was assessed by reconstitution of human bone and thymus fragments implanted into SCID mice as well as by various in vitro culture systems. The more primitive, multipotent HSCs were found to be $CD34^+$ Lin^- Thy 1^+ and $CD45RA^-$; these were able to repopulate both the thymus and the bone marrow fragments. However, a $CD34^+$ Lin^- Thy 1^- $CD45RA^+$ fraction, considered to be the progeny of HSCs, lacked ability to repopulate bone marrow, to maintain $CD34^+$ cells in culture, or to form erythroid cells, but the fraction had enhanced T-progenitor activity in the thymus fragment reconstitution assay. It would be of interest to know if these cells contain TdT, since a $CD34^+$ TdT^+ bone marrow population has been described by Gore et al (28) and considered to represent early lymphoid precursors, although functional assays were lacking. Recent extensions of these studies by Galy and colleagues (AHM Galy, personal communication) have led to the identification of a "lymphoid progenitor" population with a capacity to form T cells, B cells, natural killer (NK) cells, and interdigitating dendritic cells (DC), but not to form normal myeloid, erythroid, or megakaryocytic cells. This suggests development within the bone marrow of a common lymphoid progenitor, if the term can be extended to include certain DC. However, clonal studies will be required to eliminate the possibility that the population is a mix of individual unipotent progenitors (either T, or B, or NK or DC committed) with identical surface phenotype. At this stage it is not clear whether mouse and human differ in their bone marrow stem cell populations, or

whether the markers and the assays used for human bone marrow have simply proven more effective at revealing lymphoid commitment.

EARLY T CELL PROGENITORS AND THYMUS SEEDING CELLS IN BLOOD

The anatomical separation of the source of stem cells from the site of T lymphopoiesis in the thymus provides a useful inbuilt segregation of two levels of T-lymphocyte development. However, the issue of whether thymopoiesis in the adult requires a continuing input of progenitors from bone marrow, or whether it is self-sustaining, has long been a point of dispute. If HSCs are injected directly into an irradiated thymus, they produce long-term reconstitution and persistence of stem cells able to form spleen colonies (29). However, this mode of HSCs entry into the thymus is obviously artificial. Donskoy & Goldschneider (5) have made a careful reexamination of the issue of thymic seeding in the adult, employing Thy-1 alloantigen-disparate parabiotic mice. They concluded that thymopoiesis is dependent on a low but continuing input of blood-borne progenitor cells, in agreement with the earlier assessment of Scollay (4)

What form of progenitor enters the thymus from the bloodstream? Until recently attempts to answer this question were indirect, relying on the characterization of the cells with thymus seeding capacity in the bone marrow or on the characterization of early T precursors in the thymus. As discussed above, the data from analysis of bone marrow is ambiguous, although it provides some good candidates for a thymic seeding role. However, even the presence of T-restricted progenitors in bone marrow does not prove they will make the transit to the thymus. On the other hand, the analysis of the earliest T-progenitors in the thymus also provides incomplete, if important, information. Within the embryonic mouse thymus there are precursors capable of forming both B cells and macrophages as well as T cells (30, 31). Within the postnatal human thymus there are $CD34^+$ cells with myeloid as well as T cell–developmental capacity (32, 33). Within the adult mouse thymus, early T-precursor populations can form as well as T cells, B cells, NK cells, and DC (34, 35). All this suggests the thymus, or at least the embryonic thymus, is seeded by some form of multipotent stem cell (36).

In the case of the adult mouse thymus, there are two arguments against seeding with a multipotent HSCs. First, although T-lineage cells are produced when pure bone marrow HSCs are injected intrathymically, so are granulocytes, which are not normally found in significant numbers in the thymus (37). Second, very few multipotent stem cells (such as cells forming spleen colonies) can

be extracted from the adult thymus itself. However, if the actual rate of multipotent HSC seeding is very low, and if they become restricted in developmental potential soon after entry, these arguments are negated. The available evidence does suggest a very low seeding rate. Studies on parabiotic mice indicate that intrathymic precursors are being replaced at a rate of only 2–3% per day (5). In addition, the number of "environmental niches" available for seeding the adult thymus has been estimated to be only around 200, most of which are occupied in a normal, nonirradiated animal (37).

It is evident that a more direct approach is required. The appropriate place to search for the progenitor cells seeding the thymus from the bloodstream is in the blood itself, and the appropriate assay for the seeding process is intravenous rather than intrathymic transfer. Rodewald et al (38) have responded to this challenge by characterizing the T-progenitor populations in fetal blood at day 15.5 of gestation. In some compensation for the technical demands of handling minute samples of blood, most of the leukocytes present at this age are progenitor cells. Two progenitor populations with thymic reconstitution activity were isolated. One, Thy-1^- c-kit^+, was able to colonize bone marrow as well as thymus and was a multipotent HSC because it generated T cells, B cells, myeloid cells, and erythroid cells. The other, Thy-1^+ c-kit^{lo}, was a T-restricted progenitor because it was unable to reconstitute bone marrow or to form B cells or other hemopoietic lineages. No common lymphoid-restricted progenitor was detected. Although these experiments still do not directly show seeding from the blood to the thymus in the embryo itself, this seems likely since these two different T progenitors at different stages of development are in the bloodstream and have a demonstrated capacity for thymus homing. These experiments also demonstrate that T-lineage commitment can occur before entry into the thymic environment. Similar experiments are now required to characterize the cells in the blood of adult mice and humans that have thymus seeding ability.

EARLY T PROGENITORS IN THE THYMUS

Adult Mouse Thymus

The search for early T precursors in the adult murine thymus began with a subdivision of the 2–5% "triple negative" ($CD3^-4^-8^-$) population. Within this population it was evident that expression of the α chain of the IL-2 receptor (CD25) by about half the cells marked an important stage of development (39, 40), although the function of this low affinity chain is still obscure. However, even the $CD25^+3^-4^-8^-$ subset is heterogeneous; the additional markers CD44 (Pgp-1) and c-kit (the receptor for stem cell or steel factor) are required to segregate the individual steps of development (41, 42). Godfrey, Zlotnik and

colleagues (42, 43) have recently characterized two $CD25^+$ populations on the mainstream pathway leading to $\alpha\beta$ T cells, namely $CD3^-4^-8^-25^+44^+$ c-kit^{hi} and $CD3^-4^-8^-25^+44^-$ c-kit^{lo} (populations C and D of Figure 1 respectively). The former has $C\beta$ genes in germline state whereas the latter is extensively rearranged (42); using a polymerase chain reaction (PCR) assay, we have now shown the same applies for the $V\beta2$, $D\beta2$ and $J\beta2$ genes (L Wu, unpublished). Rearrangement of the TCR β chain therefore occurs quite abruptly within the $CD25^+$ subset, not gradually along with surface marker changes as we had previously deduced (40). Further development after β-chain rearrangement is marked by loss of CD25 (population E of Figure 1), then upregulation of CD4 and CD8 and rearrangement of the TCRα chain (reviewed in 43, 44). Control of exit from the $CD25^+$ stage is probably mediated by an association of the TCR-β chain with the newly discovered pTα chain and the CD3 complex (45).

Other, numerically minor, T cell lineages developing in the thymus appear to branch off at about the point of CD25 expression (43) (Figure 1). One is the

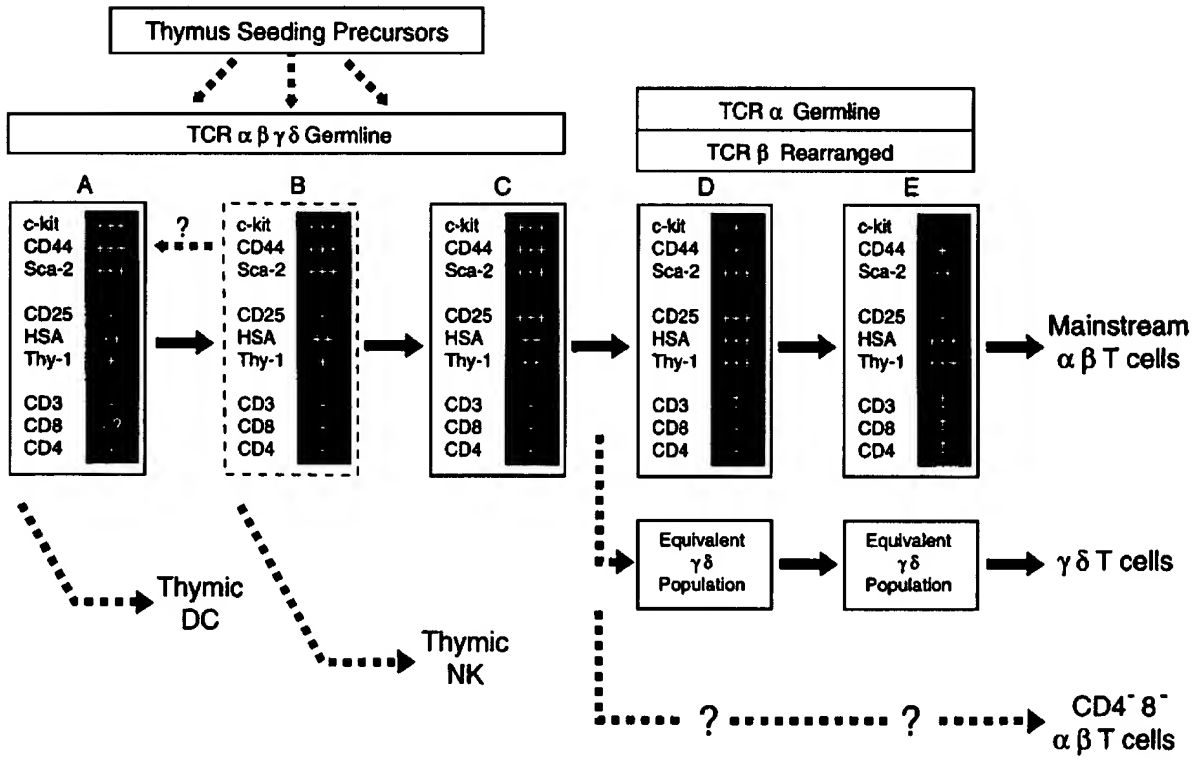

Figure 1 Populations of early T precursors in the mouse thymus. Only populations *A*, *B*, and *C*, together around 0.1% of all thymocytes, are early precursors without TCR gene rearrangement. In the adult thymus populations *A* and *C* are present, with a probable trace of *B*. In the early embryonic thymus population *B* is more prominent than population *A*. Arrows indicate developmental pathways, the solid ones probable, the broken ones possible.

$CD3^+4^-8^-$ TCR$\alpha\beta^+$ T cells, which can be produced from the $CD25^+$ subset on culture with IL-7 (43). The other is the lineage of $\gamma\delta$ T cells which develop in and exit from the adult thymus. These can be generated from $CD3^-4^-8^-$ cells that are $CD25^+$, as well as from those that are $CD25^-$ 44^{lo} HSA^{hi} Thy 1^{hi} (43, 44). Most of the $\gamma\delta$-expressing T cells in the adult thymus are proliferating HSA^{hi} Thy 1^{hi} cells, and those that exit the adult thymus also have this phenotype (47). As far as surface phenotype is concerned, these $\gamma\delta$ T cells therefore pass through developmental stages indistinguishable from populations *D* and *E* of the $\alpha\beta$ T lineage in Figure 1. An important issue, however, is whether these cells were precommitted to the $\gamma\delta$ lineage at a much earlier stage. Dudley et al (48) have recently reported that many $\alpha\beta$ T cells show evidence of failed attempts at δ and γ gene rearrangement, suggesting a very late and stochastically determined choice of lineage after rearrangement has commenced. Populations *D* and even *E* may therefore be not just similar but identical for the $\alpha\beta$ and $\gamma\delta$ lineages.

The $CD3^-4^-8^-25^+44^+$ c-kit^+ population of the adult mouse thymus (*C* of Figure 1) thus fulfils our criteria for an early T-precursor population. A different and apparently earlier T-precursor population (*A* of Figure 1) was first isolated in our laboratory (49), then by other groups (35, 43). This population expresses moderate levels of CD4, sufficient for the population to be lost when preparing $CD3^-4^-8^-$ thymocytes by depletion procedures, and it contains mRNA for CD4 (49). The population also shows low surface staining for CD8 (H Nakauchi, personal communication; L Wu, unpublished), but to date we have not found CD8α mRNA in these cells (J Ismaili and L Wu, unpublished). There is no obvious function for the premature CD4 expression by this so-called "low CD4" precursor, and it loses CD4 as it progresses to the $CD25^+$ stage. A similar population, but lacking CD4, is found in the embryonic thymus (see below) and can be detected by surface markers at trace levels in the adult (*B* of Figure 1). The "low CD4" precursor (*A* of Figure 1) has Cβ, Cγ, Vβ2, Dβ2 and Jβ2 genes in germline state (49; L Wu, unpublished), although it already expresses the recombinase activating genes RAG-1 and RAG-2 (50; L Wu, unpublished). The surface phenotype of this population is more akin to the bone marrow HSCs than later T-lineage precursors, being low for Thy 1 and high for Sca-1, CD44 and c-kit. However, it differs from bone marrow HSCs in expressing Sca-2. This surface molecule is a member of the Ly 6 family and is equivalent to MTS 35 or TSA-1 (51). Its function is unknown although antibodies against the molecule will disrupt $\alpha\beta$ T cell development in fetal thymus organ cultures (52).

Although these minute populations of early T precursors consisting of only 0.1% of thymocytes (*A*, *B* and *C* of Figure 1) are as close as has been obtained to the cells seeding the thymus from the bloodstream, they are probably still too numerous to be themselves these original immigrants. They are likely to

represent their expanded and possibly differentiated progeny. Nor is it clear that the cells seeding the thymus must progress via the *A* to *B* to *C* precursor sequence of Figure 1 en route to T cells, although this is the most logical progression. Entry could be at several points if the cells seeding the thymus themselves differ in maturation state. In addition, the "low CD4" precursor population could represent oligopotent precursors normally branching into a different lineage (see below), but recruited back into T cell development in the exceptional environment of the irradiated thymus used for T-precursor assays.

The phenotypic similarities of these early T precursors in the thymus to bone marrow HSCs prompts the question of their commitment to the T lineage. To date we have found no lineages other than $\alpha\beta$ and $\gamma\delta$ T cells being derived from the $CD3^-4^-8^-25^+$ population (D of Figure 1); this now needs checking by more sensitive procedures and with separate isolation of the earlier $CD44^{hi}$ c-kit^{hi} subset. However, the "low CD4" precursor population as a whole is clearly oligopotent. As well as forming $\alpha\beta$ and $\gamma\delta$ T cells on seeding the thymus, it produced typical small $B220^+$ s-Ig^+ B lymphocytes if injected intravenously (34). However, it formed very few spleen colonies and produced no detectable donor-derived myeloid cells (Mac-1^+ or Gr-1^+) when injected into Ly 5-disparate irradiated recipients, although a very low incidence of myeloid colonies was obtained in culture (34). Matsuzaki et al (35) have confirmed these findings, showing that the Lin^- Thy 1^{lo} c-kit^{hi} thymocytes are capable of forming as well as T cells, B cells, and NK1.1^+ cells (presumed NK cells). Thus, this population has many of the features expected of a common lymphoid-restricted precursor. It was therefore surprising when work in our laboratory revealed it also served as a precursor of DC on transfer to irradiated recipients, without concomitant formation of macrophages or granulocytes (53, 54). Although the yield of DC was low compared to T cells (1 per 2000), this is the normal ratio in the thymus, so all thymic DC could derive from the "low CD4" precursor population. Either some residual myeloid activity persists in the thymic precursor population, or a particular subclass of thymic DC is of lymphoid rather than myeloid origin.

Although this oligopotent, "low CD4" early thymus precursor population is remarkably homogeneous by surface marker analysis, there is no direct evidence that all these precursor activities derive from one cell, or even that most of the cells have T-precursor activity. Attempts to measure T-precursor frequency in this and other thymus populations (by limit-dilution repopulation of fetal thymus organ cultures) suggest a very low incidence of active cells (55). However, this may reflect a limited efficiency in seeding the right microenvironment or a requirement for limiting accessory cells. It is possible that the "low CD4" precursor population includes a variety of unipotent precursors of

identical surface phenotype, all derived from a multipotent form of HSC that seeds the thymus in low numbers, but then undergoes rapid differentiation and lineage restriction. The types of restricted precursors needed within the thymus might then be selectively expanded. New clonal assays are required to distinguish these various models.

Fetal Mouse Thymus

As discussed above, there is strong evidence that the embryonic thymus is seeded by and retains some multipotent stem cells (30, 31, 38), although this does not exclude the entry of T-committed precursors as well (38). Are the early intrathymic T precursors derived from these seeding cells different from those in the adult? With Antica & Scollay (56), we have sought an equivalent of the "low CD4" precursor (*A* of Figure 1) in the embryonic thymus. At day 14–15 of embryonic life no such population expressing significant levels of CD4 could be discerned, but a small population of $CD3^-4^-8^-25^-$ thymocytes expressing all the other markers (resembling *B* of Figure 1) was detected; this population gradually acquired CD4 until by day 18 it resembled the adult low CD4 precursor. However, in contrast to the adult precursor and to other precursors in the embryonic thymus, it proved very inefficient at repopulating an irradiated adult thymus (56). Hozumi et al (57) have isolated from the day-15 embryonic thymus a similar $CD3^-4^-8^-$ population that was $CD44^+$ c-kit^+ Thy 1^{lo} (resembling population *B*, Figure 1), and they have shown that, like the adult "low CD4 precursor", it has V, D and J β TCR genes in germline state, while already expressing RAG-2. However, they found that this population did have T-precursor activity, in a fetal thymus organ culture system. This suggests that these very early embryonic T precursors differ from those in the adult in requiring an embryonic environment for T-lineage development. This possibility now needs testing in side-by-side repopulation assays, using adult and embryonic precursors isolated in exactly the same way.

A further characteristic of embryonic thymus early T precursors, and of most thymocytes found at 14–16 days of embryonic age, is their expression of the low-affinity Fc receptors for IgG (FcγRII/III) (58). This is a characteristic marker of NK cells, although these thymocytes lack other NK markers and lack NK function. Such Fc receptors are not detected on adult thymus "low CD4 precursors" (L Wu, unpublished). Rodewald et al (58) have shown these early $CD3^-4^-8^-$ FcγRII/III$^+$ thymocytes are precursors of $\alpha\beta$ T cells when transferred into an irradiated thymus, but that they produce mature, functionally active NK cells when transferred intravenously. Likewise Brooks et al (59) have demonstrated that the majority of day-14 embryonic thymocytes can develop into mature NK cells in cultures containing IL-2. Thus there is strong evidence, although not as yet a formal clonal proof, that in the embryo T and NK cells

have a common precursor. This capacity to form NK cells appears to be retained for some time by these embryonic thymus early T precursors, even if it is not used in the thymic environment.

Human Thymus

The lack of data on the TCR-gene rearrangement status of early human thymocyte subpopulations limits our ability to delineate the early T precursors. Until recently lack of a suitable thymus reconstitution assay was also a limitation, which the SCID-hu mouse system (26) and the development of human T precursors in human fetal thymus organ cultures (60) have now largely overcome. Deducing a developmental sequence by direct analogy with the murine pathway is complicated by the absence of CD25 as a clear signpost of the stage of TCR gene rearrangement, as in the mouse. However, there are plentiful surface markers in the human system, and more use has been made of intracellular markers such as TdT or cytoplasmic CD3 expression. Most of the developmental pathways proposed to date have been deduced rather than experimentally derived, by assuming a sequential loss of markers associated with bone marrow HSCs (e.g. CD34) and sequential gain first of early T cell markers (e.g. CD7, CD2, CD5, CD1), then of late T cell markers (CD4, CD8, CD3) (61–63). Some of these steps have now been verified by testing precursor-product relationships either in culture or in the new thymus reconstitution systems.

The earliest intrathymic T precursors in the human thymus would be expected to express CD34, found on HSCs and most early precursors. Indeed a proportion of $CD3^-4^-8^-$ thymocytes are $CD34^+$, and only these have effective T-precursor activity (64). It has been argued that the T-lineage marker CD7 appears even before thymus seeding, since some bone marrow and fetal liver cells are $CD34^+$ $CD7^+$; however, many of these may be developing into NK cells (65). Nevertheless, the likely earliest human thymocytes are $CD34^+7^+$, and the majority of these express cytoplasmic CD3, indicating T-lineage orientation (61–63). CD2 is also present on the earliest putative human thymus T precursors, although at reduced levels (61–63). Although CD5 also is claimed to appear very early (62), a population of $CD34^+$ $CD5^-$ precursors has been described (66); CD5 expression may mark a downstream precursor. CD1 expression marks a still later developmental step (61–63). As in the murine thymus, the early precursors express CD44 (33, 61–63). However, the earliest detectable putative precursors in the infant or even in the young adult human thymus do not express CD4 (63), in contrast to the postnatal and adult mouse, but as in the early mouse embryo. Overall the earliest T precursors in the human thymus probably have the surface phenotype $CD3^-4^-8^-$ $34^+44^+7^+2^{lo}5^-1^-$.

The human thymus, both pre and postnatal, contains precursor cells within the $CD3^-4^-8^-$ fraction able to generate lineages other than T cells (32, 33, 66).

The central question is whether these share a common precursor with T cells, other than a multipotent HSC. NK cells have been shown to develop from $CD3^-4^-8^-$ thymocytes, and the presence of CD3ϵ and δ proteins within fetal NK cells strongly supports a common T/NK precursor (67). However, Sanchez et al (66) found that the majority of NK precursors and NK cells in the $CD3^-4^-8^-$ fraction are $CD34^-56^+5^-$ and without T-precursor function. Nevertheless, an earlier common T/NK precursor may well exist. The myeloid potential of $CD3^-4^-8^-$ thymocytes (30) has been reexamined recently by Marquez and colleagues (33). By following the growth and differentiation of $CD34^+3^-4^-8^-$ thymocytes cultured in the presence of IL-7, they observed the simultaneous development of T-lineage cells (small $CD4^+8^+7^+44^-$ DR^-) and non-T cells consisting of both monocytes and DC (large $CD4^+8^-7^-44^+$ DR^+). Both pathways progressed via $CD1^+4^+$ intermediate stages, but these intermediates were separable by CD44 expression. This recalls the generation of both T cells and DC from the adult mouse low CD4 precursor (53). However, these cultures may have included two independent developmental sequences, derived from separate $CD34^+$ precursors which happen to have surface antigen features in common. Once again a clonal approach will finally be needed to determine if the early T precursors also have DC or myeloid developmental potential.

Extrathymic T Cell Development

A significant proportion of all T cells, especially those found between the epithelial cells of the gut mucosa, do not develop within the thymus. These have been well reviewed recently (68) and are not considered in detail here. Some of the T-lineage precursors found in bone marrow or in blood may be destined for such extrathymic development, rather than for thymus seeding. Although there is evidence for TCR-gene rearrangement within the gut epithelium itself, neither the major sites of extrathymic T cell expansion and differentiation nor the nature of the precursor cells involved is clear at present. This is an area demanding further investigation.

THE CONTROL OF EARLY T-PRECURSOR DEVELOPMENT

Although it is clear from the preceding survey that our ability to identify and order in sequence the earliest steps in T cell development is still limited, enough is known to begin asking how the processes are controlled. These multiple steps in T cell development are determined by changes in the expression patterns of lineage-specific genes and, downstream from the area of this review, by sequential rearrangement of TCR genes. These in turn are known to be controlled

at the DNA level by certain transcription factors, controlled in the cytoplasm by signal transduction molecules including a number of tyrosine kinases, and controlled at the cell surface by receptors for cell interaction molecules and cytokines. We consider recent progress only in those regulatory molecules that clearly have a selective impact on early stages of the T lineage.

Cytokines and Cytokine Receptors

Of the cytokines that have been proposed to control early T cell development, only IL-7 has so far been shown to have an essential role. IL-7 stimulates the proliferation of immature thymocytes and mature T cells, as well as B-cell progenitors, through the interaction with its high-affinity receptor (IL-7R) (69, 70). Its action on $CD3^-4^-8^-$ thymic precursors may be more to maintain viability and function than directly to induce proliferation (71). It promotes rearrangement of TCR β and γ chain genes in fetal thymus and liver (72, 73), although it is not clear whether this is a direct effect or a consequence of increased survival and proliferation. Recent studies by Peschon et al (74) on mutant mice genetically deficient in the IL-7R have revealed a profound reduction in thymic and peripheral lymphoid cellularity. Both T cell and B cell development are blocked at an early stage. Thymocyte development is blocked, although not completely, within the $CD3^-4^-8^-$ population prior to acquisition of CD25 and prior to TCR β chain gene rearrangement, possibly at the "low CD4" precursor stage. Since in the normal thymus an early expansion of precursors precedes acquisition of CD25 and TCR-gene rearrangement, this early T-precursor expansion may be the process dependent on signals from the IL-7R. Whether IL-7R functions alone or in concert with other cytokines remains to be established.

Nonreceptor Protein Kinases

The nonreceptor protein tyrosine kinase $p50^{csk}$, predominantly expressed in adult mouse thymus and spleen, functions as a regulator of signal transduction via src-family kinases including $p56^{lck}$ and $p59^{fyn}$ (75, 76). Since $p56^{lck}$ and $p59^{fyn}$ play important roles in lymphocyte development (77, 78), $p50^{csk}$ may contribute to these regulatory circuits. Recent studies by Gross et al (79) on gene targeting of $p50^{csk}$ have shown that although $csk^{-/-}$ progenitors colonize the developing thymus, T cell differentiation is blocked at an early stage, as is that of B cells. $Csk^{-/-}$ ES cells fail to develop into adult lymphocytes in chimeric mice, whereas they are able to support the development of other hemopoietic lineages (79). Therefore, the expression of $p50^{csk}$ is selectively required for the maturation of early lymphoid progenitors, although it is not clear whether this regulatory function is via modulation of $p56^{lck}$ or via a different circuit. Nor is it clear whether $p50^{csk}$ acts before or after the stage of TCR β-gene rearrangement.

Disruption of Normal Development by a CD3ϵ Transgene

Another system for disrupting the normal control of early T cell development has been reported by Wang et al (80), who have introduced a human CD3ϵ transgene into mice. Mice with a high number of transgene inserts had no mature T cells or NK cells but do have normal B cells. Thymocyte development was blocked at the $CD3^-4^-8^-44^+25^-$ stage, clearly earlier than with the recombinase deficient mice, either RAG-1 null (81) or RAG-2 null (82), where NK cells develop normally. However, with lower copy numbers of the transgene, thymocyte development was blocked at the later $CD3^-4^-8^-44^-25^+$ stage, and normal NK cells were then detected. These experiments add further support to the notion that T cells and NK cells develop from a common precursor, although separate precursors with a common developmental mechanism involving the CD3ϵ protein is another interpretation. The data indicate that cytoplasmic tail of the CD3ϵ protein is needed to disrupt development. Whether this occurs by a positive signal or because of competitive binding of an essential signal transduction molecule is yet to be determined.

Transcription Factors

Of all the transcription factors proposed to have a role in T cell differentiation only one, the Ikaros protein, has been found selective for lymphoid development. Ikaros, a zinc-finger DNA binding protein, is comprised of five zinc finger modules organized in two clusters. It was cloned on the basis of its ability to bind to the enhancer of a gene encoding an early T cell differentiation antigen, CD3δ (83, 84). During development, Ikaros mRNA is first detected in the mouse fetal liver and then in the embryonic thymus, when hemopoietic and lymphoid progenitors initially colonize these organs. In adult mice Ikaros is expressed in T cells and their progenitors as well as in early B cells (83). In addition to this restricted expression pattern of Ikaros mRNA, high affinity binding sites for the Ikaros protein can be identified in the regulatory domain of many lymphocyte-specific genes including genes coding members of the CD3-TCR complex, CD4, CD2, TdT, IL-2Rα, NF-κB, Ig heavy and light chains, and the early B cell differentiation antigen mb-1 (84). Thus, Ikaros seemed likely to be a major determinant of lymphoid lineage commitment. This was confirmed in the recent study of Georgopoulos et al (85), which demonstrated that mice homozygous for a germline mutation in the Ikaros DNA-binding domain lack both mature and immature T and B lymphocytes as well as NK cells. In contrast, the erythroid and myeloid lineages are intact or elevated in these mutant mice. The developmental block is at an earlier stage than in recombinase-deficient mice (81, 82), and perhaps still earlier than in the CD3ϵ transgenic mice (80). However, it is not yet clear whether the Ikaros gene

products control the commitment of HSCs to a common lymphoid progenitor, or whether they function on separate restricted lymphoid-lineage progenitors that utilize the same mechanism in their subsequent development. Studies on the developmental potential of HSCs isolated from the Ikaros mutant mice, and on the genetic elements downstream of Ikaros gene, should provide more insight into the early steps in commitment to the lymphoid lineage.

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